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TITLE: Activation of Central Pattern Generator for Respiration Following Complete High Cervical Spinal Cord Interruption

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CONTRACTING ORGANIZATION:

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The original hypothesis was that intraspinal inhibitory circuits (GABA- and Glycinergic) play an important role of in the control of a spinal central pattern generator (CPG) for breathing. These inhibitory spinal interneurons were hypothesized to contribute to suppression of the respiratory CPG in both intact and post-injury (high cervical transection) conditions. Adhering to the experiments outlined in our SOW, spinal respiratory neurons (cervical C3-C5 and C1-C2 levels) were characterized by their location, pattern (via extra- and intracellular recordings) and sensitivity to blockers of GABAa and Glycine receptors (GABAzine and strychnine).CPG-specific inspiratory bursts, recorded from phrenic nerves, were observed after application of GABAzine and strychnine over C3-C5 cervical segments in spinally transected and intact animals (Ghali and Marchenko, 2016). These 'spinal bursts' were not phase-locked to the supraspinal (ponto-medullary) respiratory rhythm. We recorded spinal interneurons related to the spinal respiratory CPG, a promising target for activation of breathing in tetraplegic patients. These newest findings are being prepared for publication in peer-review scientific journals and presentation at the upcoming annual meeting for the Society for Neuroscience.

15. SUBJECT TERMS
Spinal cord injury, high cervical transection, respiration, CPG, GABA, Glycine, spinal cord

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The overall objective of this new research program is to develop strategies to promote restoration of voluntary breathing in people with cervical SCI. The focus of the present proposal will be to: 1) investigate spinal inhibitory circuits controlling phrenic motoneurons (PMNs) and thus diaphragm (the primary inspiratory muscle) activity before and after acute high cervical spinal cord transection, and 2) optimize combined drug delivery (GABAa / Glycine receptors blockers) and epidural stimulation to improve treatment efficacy of respiratory disorders in patients with SCI (including weaning from artificial ventilation in tetraplegic).

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Spinal cord, spinal cord injury (SCI), C1 spinal cord transection (C1Tx), respiration, phrenic nerve, motoneurons, interneurons, GABA, Glycine, Strychnine, GABAzine, epidural stimulation

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

As outlined in the SOW the present research addresses the following two aims:
Aim1: Identify the location and neurotransmitter profile of spinal inhibitory interneurons related to respiratory motor control and initiating of spinal CPG follow C1Tx (1-14 months).
Aim2: Identify the optimal combination of intrathecally delivered blockers of fast inhibition (Glycine- and GABA-ergic) and epidural electric stimulation for improving respiratory recovery follow C1Tx (15-36 months).
For the 1st year of project Major Task 1.1 (1-12 months) was planned:
Characterize C1-C5 respiratory-related neurons and their responses to microinjection of GABAzine, Strychnine, and GABAzine+Strychnine with follow juxtacellular labeling and identification of neurotransmitter profile.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Subtask 1.1.1: Characterize respiratory-related neurons in phrenic nucleus (C3-C5) and their responses to microinjection of GABAzine, Strychnine, and GABAzine+Strychnine with follow juxtacellular labeling and identification of their neurotransmitter profile (1-6 months).
This subtask was completed. The major finding in these experiments was that the anatomical distribution of spinal interneurons at C3-C5 level was defined in detail:
1) We found a heterogenic population of respiratory interneurons at the level of the phrenic nucleus (Fig.1-5) that may be a part of a highly-organized, local network subserving phrenic motoneurons during eupnea and different respiratory disorders including spinal cord injury;
2) We also described a more precise location of late-inspiratory neurones located slightly dorso-lateral to the main phrenic nulceus – this was confirmed by monosynaptic tracing (Fig. 3). These neurons may play an important role (by recruitment) in the compensation of breathing following spinal cord injury. We also found that late-Insp interneurons are the most sensitive spinal units to GABAa and Glycine-receptor blockers (GABAzine and Strychnine, respectively) (Fig. 6-7). We conclude that these neurons are strongly inhibited during normal breathing.
3) CPG-specific inspiratory bursts, recorded from phrenic nerves, were observed after application of GABAzine and strychnine over C3-C5 cervical segments in spinally transected animals (Fig. 11, Ghali and Marchenko, 2016). We recorded multi-unit activity of spinal interneurons related to the spinal respiratory CPG (Fig. 11), a promising target for activation of breathing in tetraplegic patients.
Subtask 1.1.2: Characterize respiratory-related neurons in upper respiratory group (C1-C2) and their responses to microinjection of GABAzine, Strychnine, and GABAzine+Strychnine followed byjuxtacellular labeling and identification of their neurotransmitter profile (months 7-12).
This subtask is 100% complete. The major findings are related to the detailed location of spinal interneurons at C1-C2 level:
1) We found a very heterogenic distribution of respiratory interneurones that are located more sparsely when compared to the C3-C5 level (Fig.8). The majority of recorded units in C1-C2 were found to be Inspiratory with tonic background activity. Some interneurons show pre-Inspiratory--Inspiratory pattern (Fig. 9) that is typical for the neurones located in the pre-Bötzinger Complex (the main medullary structure responsible for generation and maintenance of respiratory rhythm). It is possible that these units are involved in generation of respiratory-like activity after spinalization.
Subtask 1.1.1.
A total of 39 experiments were completed and recording of respiratory motor- and interneurons (n=137) from C3-C5 cervical segments were made. Motor- and interneurons were identified by the presence or absence of antidromic response to phrenic nerve stimulation (Fig. 1). These neurons were catergorized as follows (Fig. 2):

- Inspiratory (Insp) (n=43) (34 units were identifired as motoneurons) (Fig. 4A)
- Inspiratory interneurons with tonic background activity (Insp+BG) (n=27) (Fig. 4B)
- Late-inspiratory (Late-Insp) (n=20) (14 units were identified as motoneurons) (Fig. 4C)
- Inspiratory-expiratory phase-spanning interneurons (Insp-Exp) (n=9) (Fig. 4D)
- Full expiratory (Exp) interneurons (n=17) (Fig. 4E)
- Augmenting expiratory (E2) interneurons (n=15) (Fig. 4F)
- Decrementing expiratory (E1) (n=8) (Fig. 4G)
- Inspiratory wide phase-spanning (Insp Wide Phase-Span) (n=6) (Fig. 4H)

Topographical analyses of the most reperesenative populations recorded units (inspiratory motoneurons and Insp+BG intreneurons) reveals to disctinc group of inspiratory motoneurones located ventro-medial (inspiratory, black circles, Fig. 3) and ventrolateral (late-Inspiratory, red pentagons, Fig. 3), accordingly (higlighted by yellow oval on C4-C5 panels of Fig.3). This bymodal distribution of phrenic motoneurones was confirmed by retrograde monosynaptic of different tracers (Fast Blue - see Fig.3B, Texas red and Cholera Toxin subunit B) (preiliminary data of Bezdudnaya, Lane and Marchenko experiments). Also it was found, that Insp+BG interneurones located mostly dorso-medial to motoneuron pools (otlined with pink line areas on panels C3-C5 of Fig.3). However, some of them were found in vicinity with motoneurones population (see Fig.3A for labeled Insp+BG inetrneuron[Streptavidin-Alexa-610 was used for neurobiotin detection]).

Fig.1: Identification of motoneurons (A&B) and interneurons (C) by antidromic stimulation of phrenic nerve

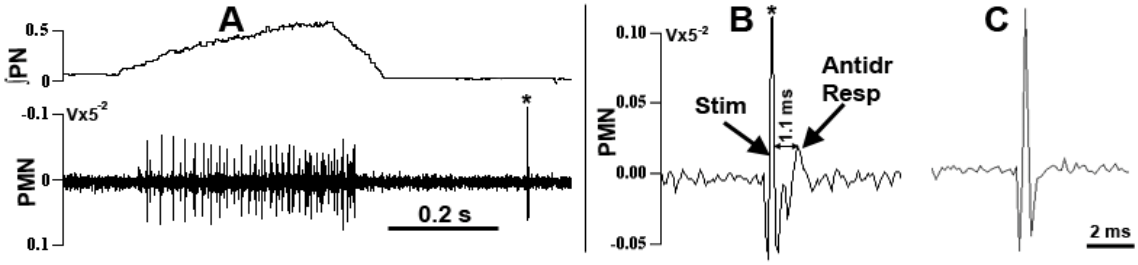


Fig.2: Distribution of respiratory-related neurons in the middle cervical (C3-C5) segments

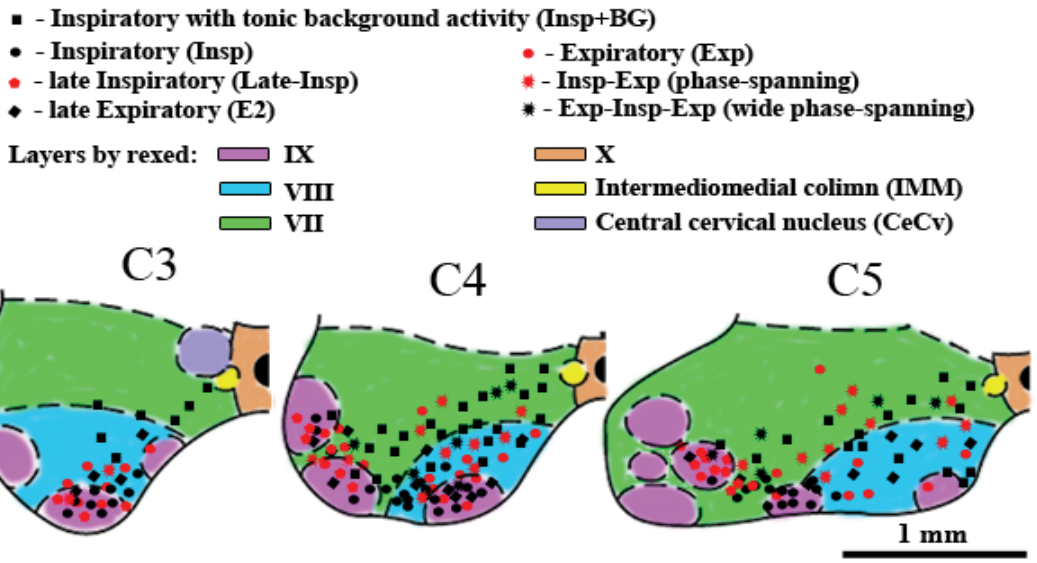


Fig.3: Labelling (A) and distribution of inspiratory neurons with background activity (C3-C5, Insp+BG: black rectangles) relative to motoneurons (B and C3-C5, Insp: black circles, Late-Insp: red pentagons)

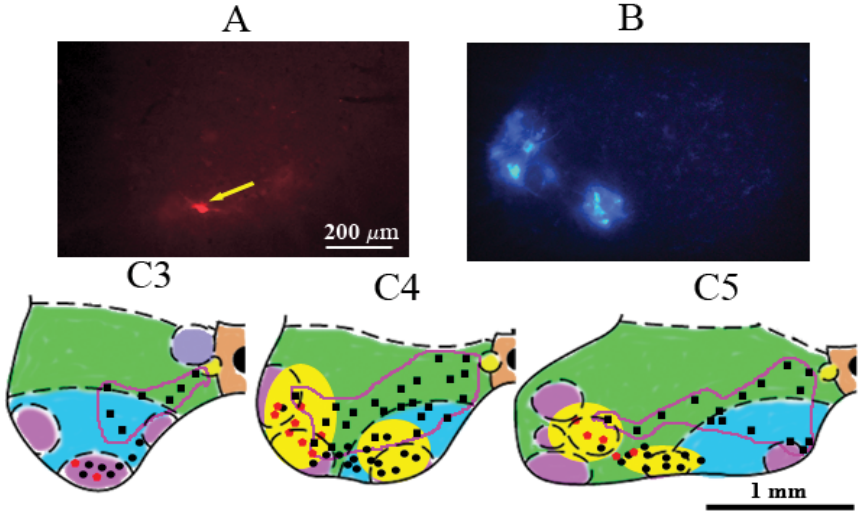


Fig. 4-A: Inspiratory (Insp) unit

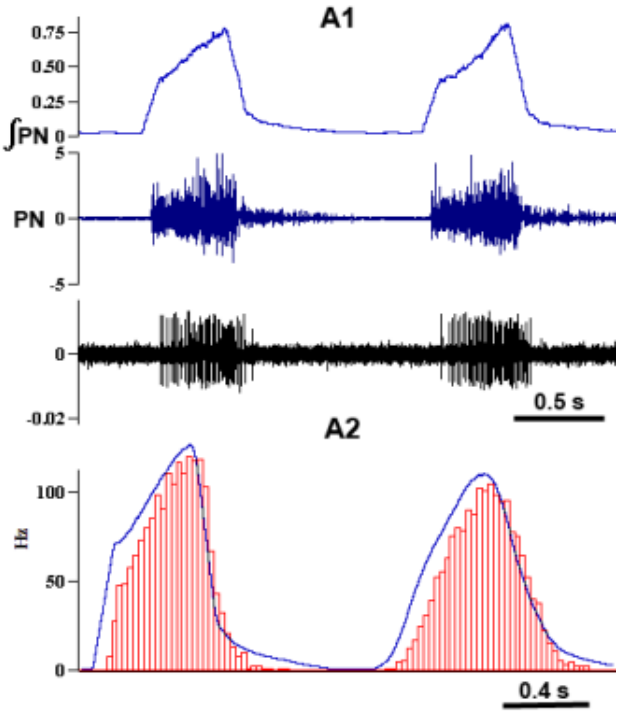


Fig. 4-B: Inspiratory unit with background activity (Insp+BG)

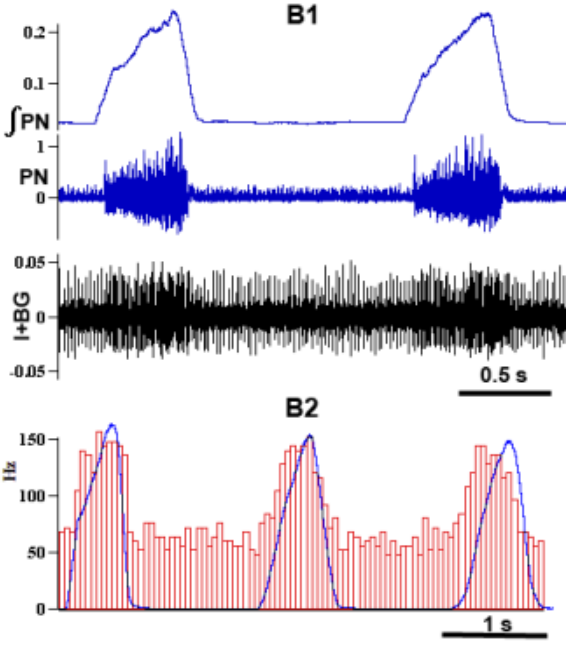


Fig. 4-C: Late-Inspiratory (late-Insp) unit

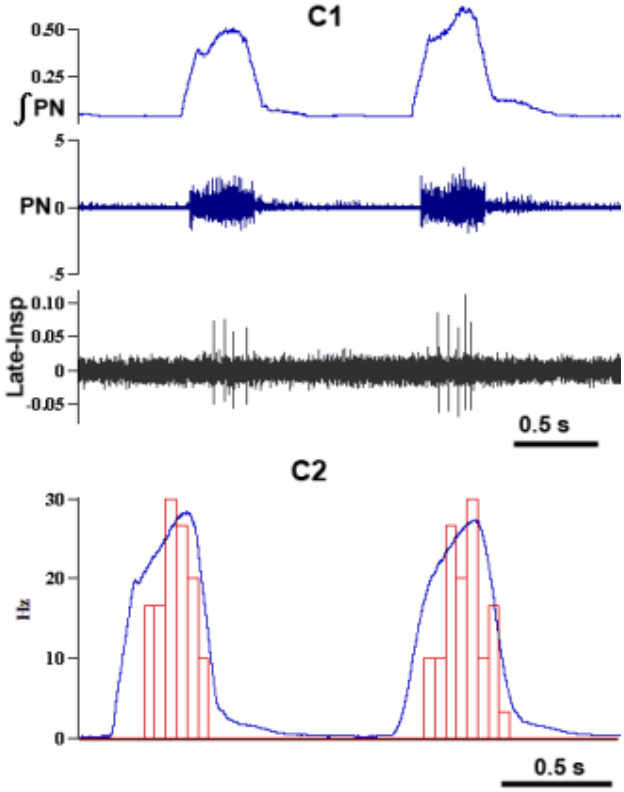


Fig. 4-D: Ispiratory-Expirarory (I-E) phase-spanning unit

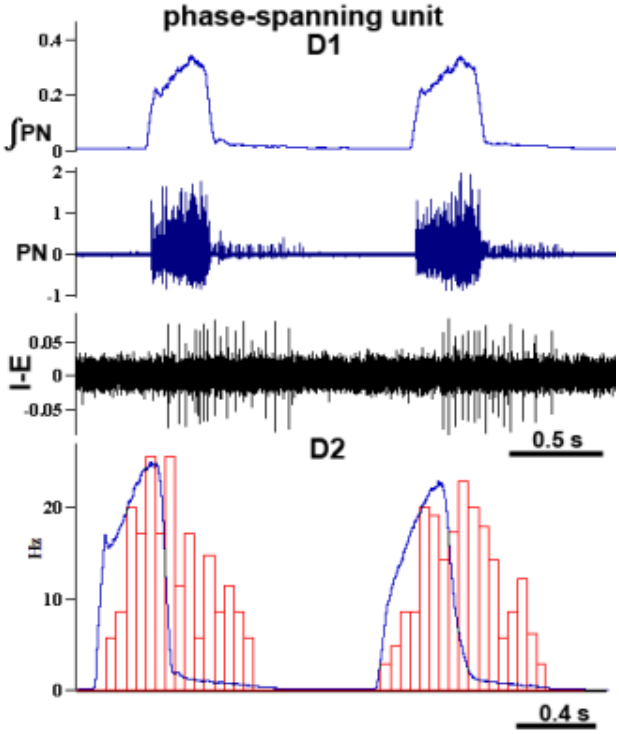


Fig. 4-E: Full-Expiratory (E) unit

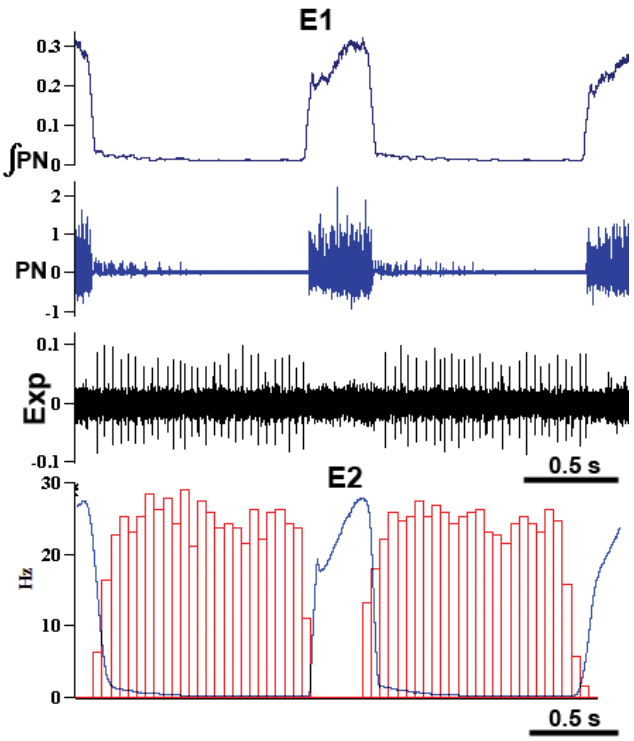


Fig. 4-F: Expiratory-Augmenting (E2) unit

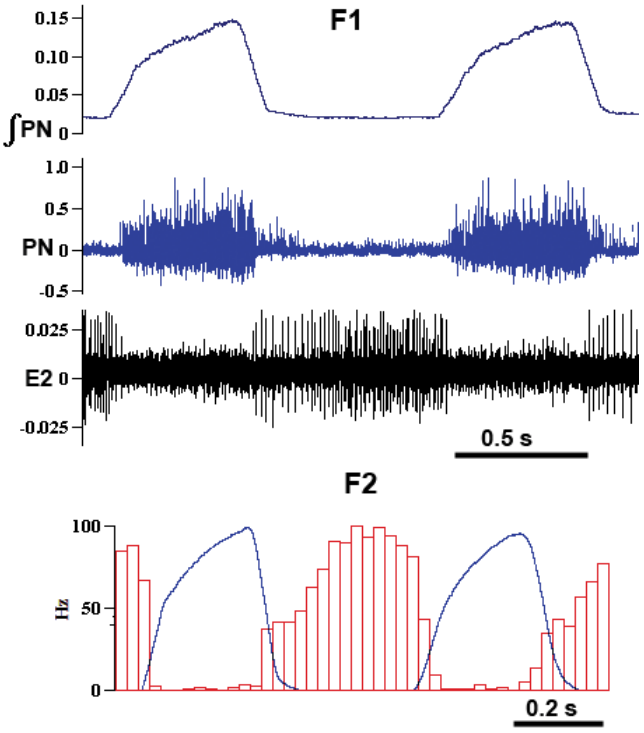


Fig. 4-G: Expiratory-Decrementing (E1) unit

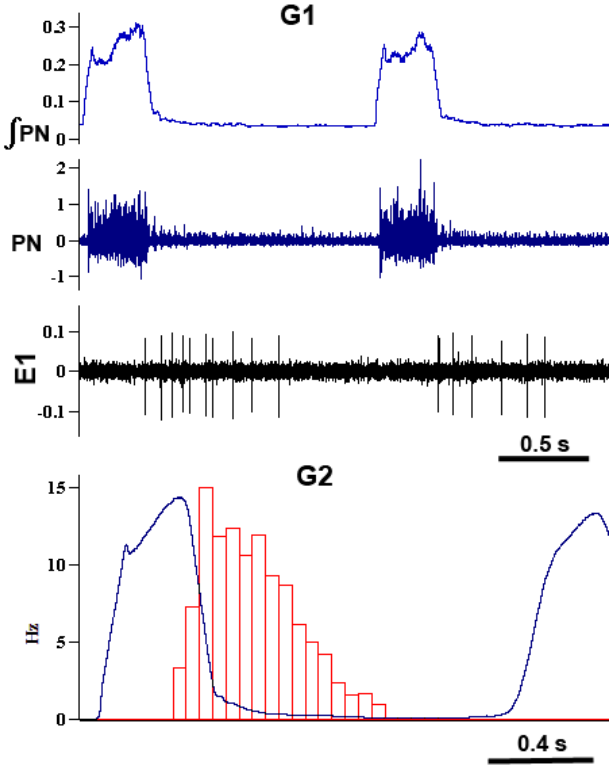
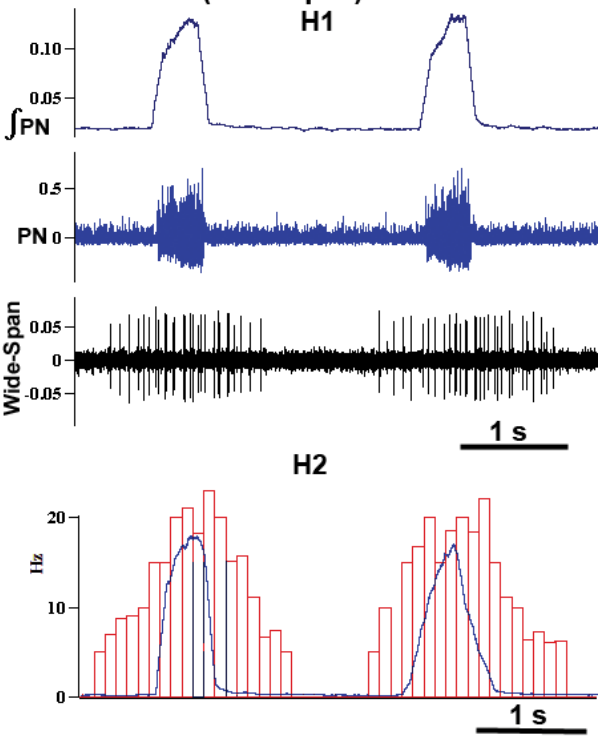


Fig. 4-H: Inspiratory Wide-Spanning (Wide-Span) unit



Patterns of spinal respiratory neurons shown in Fig. 4A-H: upper panels (A1-H1) contain both raw and integrated unit recordings of phrenic nerve (PN activity); low panels (A2-H2) demonstrate post-stimulus histograms (obtained from onset of phrenic nerve discharge) of unit frequency distribution.

Seven inspiratory motoneurons and three interneurons (Insp+BG) were successfully intracellularly recorded (Fig. 5A). GABAzine (high selective antagonist of postsynaptic GABAa receptors, 4 mM) and Strychnine (STR, antagonist of glycine postsynaptic receptors, 4 mM) were applied to 61 cells juxtacellular with microiontophoresis (+20 nA, 2 min) using multibarrel Carbostar electrode followed by labeling (2% Neurobiotin, +10 nA, 30 min). In response to application of antagonists only 3 of 22 Insp+BG cells showed significant changes in firing frequency while all tested late-Insp units (20/20) showed an altered firing pattern to become early-inspiratory units with tonic background activity

Fig. 5-A: Intracellular record from inspiratory motoneuron

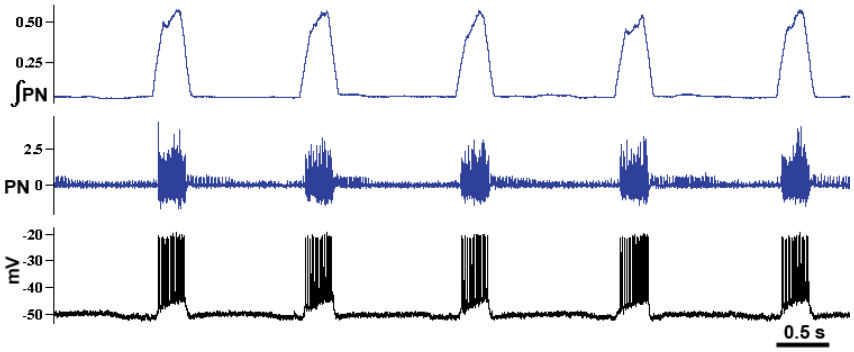


Fig.6. Response of Insp+BG spinal neuron to Strychnine application

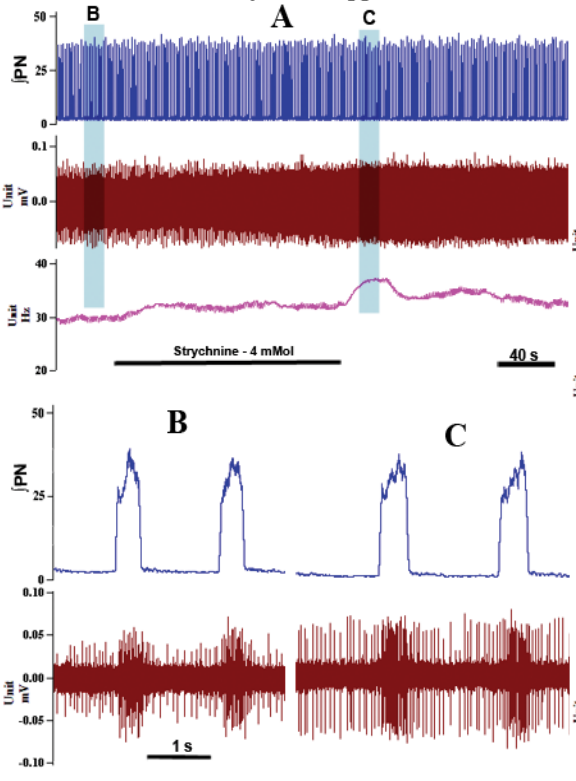
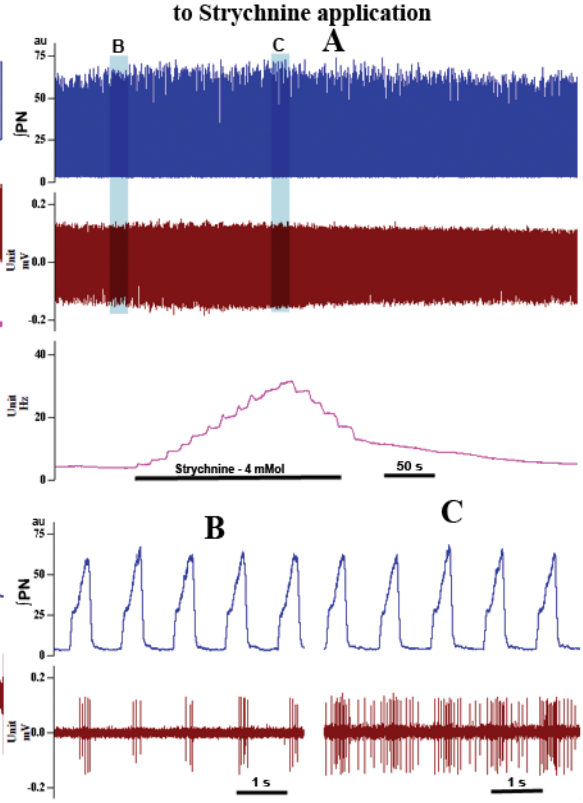


Fig.7. Response of Late-Inspiratory spinal neuron to Strychnine application



Subtask 1.1.2.

A total of 72 (10 – in 1st quarter, 29 – in 2nd quarter, 19 – in 3d quarter and 14 – in 4th quarter) experiments were completed with recordings from respiratory-related neurons (n=104) in C1-C2 cervical segments. All recorded units were identified as interneurons by the absence of an antidromic response to phrenic nerve stimulation (Fig.1). These neurons were categorized as follows (Fig. 8):

- Inspiratory (Insp) (n=8)
- Late-inspiratory (Late-Insp) (n=10)
- Expiratory (Exp) interneurons (n=15)
- Augmenting expiratory (E2) interneurons (n=9)
- Inspiratory interneurons with tonic background activity (Insp+BG) (n=2)
- Inspiratory-expiratory phase-spanning interneurons (Insp-Exp) (n=14)
- Inspiratory wide phase-spanning (Insp Wide Phase-Span) (n=11)
- Exp-Insp phase-spanning (pre-I-Insp interneurons) (n=12).

Topographical analyses of recorded units in C1-C2 revealed a dense concentration of these cells in the medial area of Rexed Layer VII. The majority of recorded units (25 of 104 cells) in C1-C2 were found to be Insp+BG interneurons. Additionally, a new class of interneurons, which fire before and during inspiration (Exp-Insp phase-spanning), were characterized in C1-C2 segments (n=12). Neurobiotin was used to label cells as demonstrated in Fig. 8 ('C2-Neurobiotin' panel). The firing pattern of these units is very close to medullary pre-Bötzinger pre-I-Insp neurons – the main kernel generating respiratory rhythm (Fig. 9). These neurons may relay/amplify the supraspinal drive to respiratory motoneurons during normal respiratory behavior and may play an important role in initializing a spinal CPG for breathing after high cervical spinal cord disruption. We will pay close attention to these cells in future recordings, with the aim of determining whether they represent an important therapeutic target post-SCI. Neuropharmacological experiments show the high sensitivity of Late-Insp neurons to GABAzine and Strychnine (Fig. 10), as was demonstrated for C3-C5 level.

Fig.8: Distribution of respiratory-related neurons in the upper cervical (C1-C2) segments

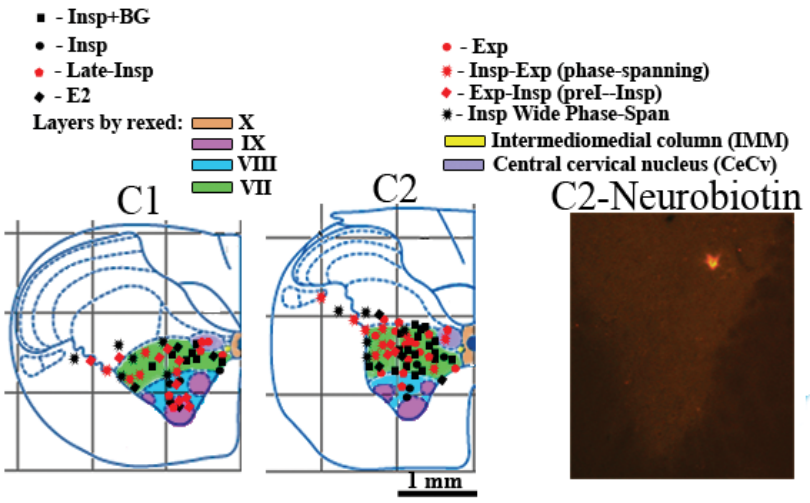


Fig. 9. pre-Inspiratory--Inspiratory (prel-Insp) unit

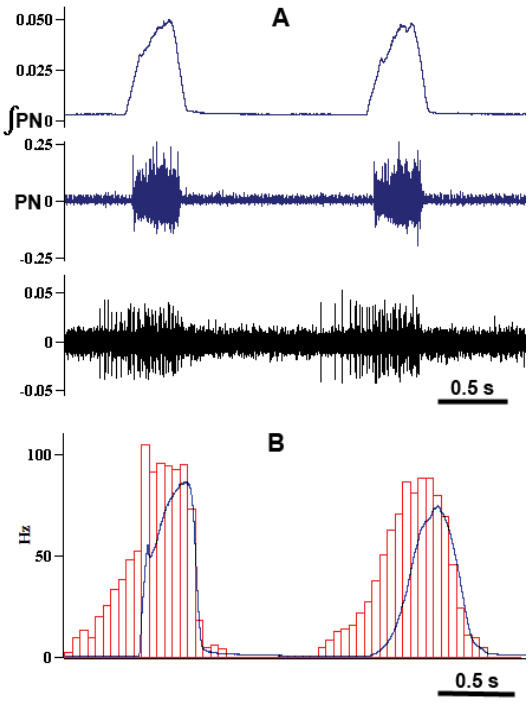


Fig. 10: Response of late-Inspiratory C2 spinal neuron to GABAzine application

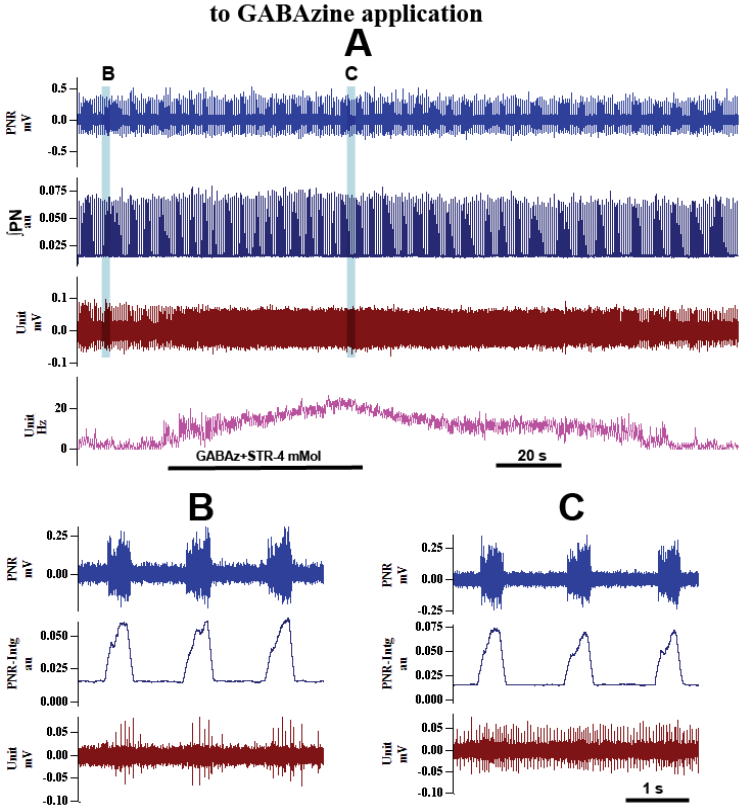
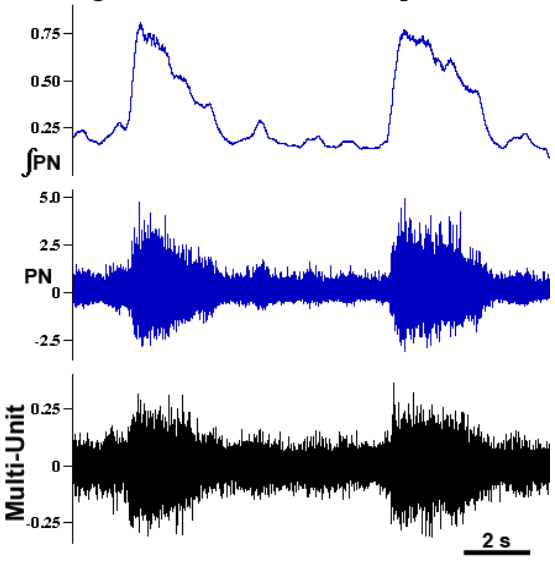


Fig. 11: Multi-Unit activity of spinal interneurones during GABAz&STR-activated spinal CPG



What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

According to the SOW we will find an optimal combination of intrathecal drug delivery (GABAzine and Strychnine) and epidural stimulation in high cervical transected animals.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal

disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

In the majority of cases people with tetraplegia are not able to breathe voluntarily, thus artificial ventilation is required. Our project is devoted to the development of treatments that harness the neuroplastic potential of spinal circuits (interneurons and motoneurons) that are capable of generating rhythmic breathing-like activity in respiratory muscles in an animal model of complete high cervical spinal cord injury. To date the role of spinal respiratory interneurons in respiratory motor pattern formation is almost ignored. The present work addresses this gap in research, bringing this clinically relevant concept to the forefront of this field of SCI research by proving the importance of intraspinal circuits in respiratory motor pattern formation.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  1. Ghali Michael George Zaki and **Marchenko Vitaliy***. Patterns of Phrenic Nerve Discharge after Complete High Cervical Spinal Cord Injury in the Decerebrate Rat. Journal of Neurotrauma. June 2016, 33(12): 1115-1127. *acknowledgement of federal support: yes.*
  2. Hormigo KM, Zholudeva LV, Spruance VM, **Marchenko V**, Cote MP, Vinit S, Giszter S, Bezdudnaya T, Lane MA. Enhancing neural activity to drive respiratory plasticity following cervical spinal cord injury. Exp Neurol. 2016 S0014-4886(16)30267-9 - InPress. *acknowledgement of federal support: yes.*

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Bezdudnaya T., Lane M. A., **Marchenko V.*** Distribution of respiratory-related neurons in C3-C5 cervical segments and their responses to blockade of GABAa and Glycine receptors in decerebrate rats. Abstract was submitted for SFN-2016 Symposia. Control/Tracking Number: 2016-S-13226-SfN. *acknowledgement of federal support: yes.*

  Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  1. Ghali Michael George Zaki and **Marchenko Vitaliy***. Effects of vagotomy on hypoglossal and phrenic responses to hypercapnia in the decerebrate rat. Respir Physiol Neurobiol. 2016 Oct;232:13-21. Was supported by Drexel University and Craig H. Neilson Founation (2014).
  2. **Vitaliy Marchenko**, Hidehiko Koizumi, Bryan Mosher, Naohiro Koshiya, Mohammad F. Tariq, Tatiana G. Bezdudnaya, Ruli Zhang, Yaroslav I. Molkov, Ilya A. Rybak, Jeffrey C. SmithPerturbations of Respiratory Rhythm and Pattern by Disrupting Synaptic Inhibition within Pre-Bötzinger and Bötzinger Complexes. eNeuro 3(2). pii: ENEURO.0011-16.2016 (2016). Work was supported by NIH grant (2010-2015, PI Rybak IA)

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Name: Vitaliy Marchenko – no changes
Project Role: PI

Name: Michael Lane – no changes
Project Role: collaborator

Name: Tatiana Bezdudnaya
Project Role: Research-Instructor
Nearest person month worked: 1.05 (35% or 4.2 months per year), $ 1,750 monthly salary
Researcher Identifier: n/a
Contribution to Project: Dr. Bezdudnaya, PhD, works at our department as instructor and has a many year strong experiences in electrophysiology and programming. She was hired on part time (35% of salary support) from Sept 1st/2015 and performed work in electrophysiological experiments and data analyses.

Name: Kristiina Hormigo (Negron before marriage)
Project Role: Research Assistant II
Nearest person month worked: 50% (or 6 months per year), $ 18,750 yearly salary
Researcher Identifier: n/a
Contribution to Project: Dr. Negron, MS, works at our department as a Research Assistant II and has a strong experience in immunohistochemistry and maintaining the lab. She was hired on part time (50% of salary support) from May 1st, 2016 and has performed work in histological experiments and data analyses.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission.

Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

1. Ghali Michael George Zaki and Marchenko Vitaliy*. Patterns of Phrenic Nerve Discharge after Complete High Cervical Spinal Cord Injury in the Decerebrate Rat. Journal of Neurotrauma. June 2016, 33(12): 1115-1127.

2. Hormigo KM, Zholudeva LV, Spruance VM, Marchenko V, Cote MP, Vinit S, Giszter S, Bezdudnaya T, Lane MA. Enhancing neural activity to drive respiratory plasticity following cervical spinal cord injury. Exp Neurol. 2016 S0014-4886(16)30267-9 – InPress
3. Bezdudnaya T., Lane M. A., Marchenko V.* Distribution of respiratory-related neurons in C3-C5 cervical segments and their responses to blockade of GABAa and Glycine receptors in decerebrate rats. Abstract: SFN-2016 Symposia. Control/Tracking Number: 2016-S-13226-SfN.